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NOTE

The Dependence of the Diffuse Reflection Coefficient of Blood on the Concentration of Red Cells

We investigate here the dependence of the diffuse reflection coefficient R of thick layers of human blood on the volume fraction of red cells. It is found that this dependence is weak and can be neglected in most cases. This conclusion is important for problems of the blood spectroscopy and optical particle sizing of red cells. The asymptotical relations of the radiative transfer theory for weakly absorbing light scattering media are used to explain this effect. © 1998 Academic Press

Key Words: **light scattering; biooptics; radiative transfer theory; red cells.**

1. INTRODUCTION

Turbid media with densely packed particles are of particular interest in colloid science, geooptics (snow, foam, soils), biooptics (blood, tissues), photography (films and photopaper), biochemistry, and other fields of modern physics and technology. This branch of light scattering optics is based on the multiple scattering theory for electromagnetic fields, presented in (1). However, it is difficult to obtain practical results in the framework of the general multiple scattering theory (especially for large particles in the optical band). Thus, in this paper we will use the radiative transfer theory (1) to describe the reflection of light from blood. The radiative transfer equation is valid only for rarefied media. However, it can be used to estimate radiative characteristics of turbid media at high concentrations of large particles as well, if one uses modified equations to relate local optical characteristics of turbid media with characteristics of single particles (2).

Generally speaking, local and global optical light scattering characteristics of close-packed media depend on the concentration and size of particles, their shape, and refractive index (2–5). However, it is a task of this paper to show that concentration effects can be neglected for some measured values.

We will use the approximate solution of the radiative transfer equation for semi-infinite media for the interpretation of the results obtained.

2. EXPERIMENT

The task of this paper is to investigate the dependence of the diffuse reflection coefficient (plane albedo) $R(\mu_0)$ on the concentration of erythrocytes (red cells) in human blood. This coefficient is defined as

$$R(\mu_0) = \frac{1}{\pi}\int_0^{\pi} \mu d\mu \int_0^{2\pi} d\psi R(\mu_0, \mu, \psi), \qquad [1]$$

where $R(\mu_0, \mu, \psi)$ is the reflection function, $\mu = \cos\vartheta$, $\mu_0 = \cos\vartheta_0$, ψ is the azimuth, ϑ_0 is the incidence angle, ϑ is the observation angle, and $R(\mu_0, \mu, \psi)$ is the bidirectional reflection function of a layer, defined as

$$R(\mu_0, \mu, \psi) = \frac{\pi I(\mu_0, \mu, \psi)}{F\mu_0}, \qquad [2]$$

where F is the density of the incident light flux perpendicular to the incident beam and $I(\mu_0, \mu, \psi)$ is the diffuse light intensity.

Blood cells carry oxygen to the body tissues and carbon dioxide away from them. They can be presented as double-concave disks with the mean diameter approximately equal to 7 μm. The edge thickness is equal to about 2 μm. Thus, the aspect ratio of these particles (diameter/edge thickness ratio) is about 3.5. In plasma blood cells stick together with their flat sides and form aggregates ("coin stacks"). Note that in the spectral range $\lambda = 0.645$–$1\ \mu$m the values of the relative refractive indices of red cells n and their absorption coefficients χ lay in the range $n = 1.055 - 1.060$, $\chi = 10^{-6} - 10^{-4}$.

Thus, red corpuscles are weakly absorbing nonspherical optically soft large particles in the visible region of the electromagnetic spectrum: $|m - 1| \ll 1$, $\alpha a \ll 1$, $ka \gg 1$, $k = 2\pi/\lambda$, where $m = n - i\chi$ is the refractive index of red cells, $\alpha = 4\pi\chi/\lambda$, λ is the wavelength of the incident radiation, and a is the average size of erythrocytes.

Measurements of the diffuse reflection coefficient were performed with the spectrophotometer with an integrating sphere, which was 200 mm in diameter. The scheme of the experiment is presented in Fig. 1 and described in (5) in more detail. Light from the source 1 passes the diaphragm 2, filters 3, modulator 4, and penetrates to the scattering layer 6 along the normal to a layer. The mirror component is reflected outside of the sphere through the input window. The reflected radiation is integrated by the sphere 5, the internal surface of which is covered by MgO, detected by the photodetector 7, and analyzed by the processing system 8 to obtain the diffuse reflection coefficient at the normal incidence (see Eq. [1]). This value is also called the plane albedo. Thus, the measurements are relative.

Since, as was mentioned before, red cells in whole (undiluted) blood form aggregates, they were washed out for disaggregation in a 0.9% solution of NaCl by the methods accepted in the hematological practice. The erythrocytes' concentration was varied by successive dilution of disaggregated blood with the maximal volume fraction c being equal to 0.68. The minimal value of c was equal to 0.08. The thickness of the scattering layer L was equal to 4 mm in our experiment. Such a layer of human blood can be considered as a semi-infinite medium because its reflection characteristics are not changed with increasing the thickness.

The results of the experiment are presented in Fig. 2 at $\lambda = 500$ and 660 nm and different volume fractions of red corpuscles. The experimental error was less than 10% (5). One can see that the reflectance at the normal incidence practically does not depend on the volume fraction of red cells. This result is interesting. Indeed, local optical characteristics of blood do depend on the concentration of particles and these dependencies are nonlinear in most of cases (2–5). Thus, there is a possibility for the cancellation of concentration effects in the value of the diffuse reflection coefficient. Let us study such a possibility in the framework of the radiative transfer theory.

3. THEORY

It is well-known fact that the reflection function of any semi-infinite multiply light scattering medium depends only on two parameters of singly

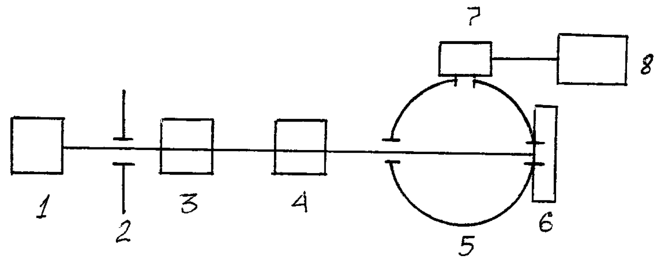

FIG. 1. The scheme of the experiment.

scattered light, namely on the probability of the photon absorption (PPA) $\beta = \sigma_{abs}/\sigma_{ext}$, where σ_{abs} and σ_{ext} are absorption and extinction coefficients, and the probability of photon scattering (PPS) in the direction specified by the scattering angle θ (the phase function $p(\theta)$). These probabilities do not depend on the volumetric concentrations (volume fractions) c at $c < 0.1$ (2–4). For larger concentrations of particles both the PPA and PPS depend on the value of c. For instance, it follows from data presented in (3) that in the case of human blood the account for the concentration of particles in the value of σ_{abs} can be done by means of the multiplyer $1 + Ac^2$, where A is approximately equal to 1 and $c < 0.8$. One can see that this dependence is rather weak. The phase function $p(\theta)$ of close-packed media is equal to the value of $p(\theta)$ at $c \to 0$, multiplied on the structure factor $S(\theta)$ (2, 6), which depends on the concentration of particles.

Thus, generally speaking, the reflection function of a semi-infinite medium depends on the concentration of densely packed particles. However, from our data it follows that this dependence for the integrated reflection function (see Eq. [1]) is rather weak. To interpret this effect, we note that the plane albedo of a semi-infinite medium does not depend on the details of the function $p(\theta)$ (7, 8) and can be calculated with the following approximate equation (7–9),

$$R(\mu_0) = \exp\left(-4u(\mu_0)\sqrt{\frac{\ln\left(\frac{1}{1-\beta}\right)}{3(1-g)}}\right), \qquad [3]$$

where $u(\mu_0) = \frac{3}{7}(1 + 2\mu_0)$ ($u = \frac{9}{7}$ in our experiment) is the escape function and

$$g = 0.25 \int_0^\pi p(\theta)\sin 2\theta d\theta \qquad [4]$$

is the asymmetry parameter. This value is close to unity for red cells. This is the one of the reasons for small values of the reflection function of blood (see Eq. [3]).

For weakly absorbing media it follows that

$$\beta \to 0,\ \ln\frac{1}{1-\beta} \to \beta,\ R(\mu_0) \to \exp\left(-\frac{4su(\mu_0)}{\sqrt{3}}\right), \qquad [5]$$

where $s = \sqrt{\beta/(1-g)}$ is the well-known similarity parameter of the radiative transfer theory (8). Thus, from our measurements it follows that the dependence of the similarity parameter s on the volume fraction of red cells c is weak as well.

Let us rewrite the value of s in the form

$$s = \sqrt{\frac{\sigma_{abs}}{\sigma_{tr}}}, \qquad [6]$$

where

$$\sigma_{tr} = \sigma_{ext}(1-g) \qquad [7]$$

is the transport extinction coefficient. For large particles ($a/\lambda \gg 1$) considered in this paper, the phase function $p(\theta)$ can be represented as

$$p(\theta) = \frac{\sigma^d_{sca}p^d(\theta) + \sigma^{GO}_{sca}p^{GO}(\theta)}{\sigma_{sca}}, \qquad [8]$$

where $\sigma_{sca} = \sigma^d_{sca} + \sigma^{GO}_{sca}$ is the scattering coefficient. Functions $p^d(\theta)$, $p^{GO}(\theta)$ account for the diffraction and the geometrical optics parts of the scattered field. The same is true for the values of the asymmetry parameter (see Eqs. [4], [8])

$$g = \frac{\sigma^d_{sca}g^d + \sigma^{GO}_{sca}g^{GO}}{\sigma^d_{sca} + \sigma^{GO}_{sca}}, \qquad [9]$$

where

$$g^d = 0.25\int_0^\pi p^d(\theta)\sin 2\theta d\theta,\ g^{GO} = 0.25\int_0^\pi p^d(\theta)\sin 2\theta d\theta. \qquad [10]$$

Thus, it follows that (see Eqs. [7], [9])

$$\sigma_{tr} = \sigma^{GO}_{sca}(1-g^{GO}) + \sigma^d_{sca}(1-g^d) + \sigma_{abs}(1-g), \qquad [11]$$

where we used the equality $\sigma_{ext} = \sigma_{abs} + \sigma_{sca}$.

For weakly absorbing media ($\sigma_{abs} \ll \sigma^{GO}_{sca}$) with large particles ($g^d \approx 1$) the last two terms in Eq. [11] can be neglected and one obtains from Eqs. [5], [6], [11]

$$R(\mu_0) = \exp\left(-4u(\mu_0)\sqrt{\frac{\sigma_{abs}}{3\sigma^{GO}_{sca}(1-g^{GO})}}\right). \qquad [12]$$

This equation can be used for the interpretation of the effect presented in this paper (see Fig. 2). Indeed, the correlation between particles influences the diffracted (partially coherent) part of the scattered electromagnetic field, dominated at small angles (6, 9). At large angles, the structure factor is about unity

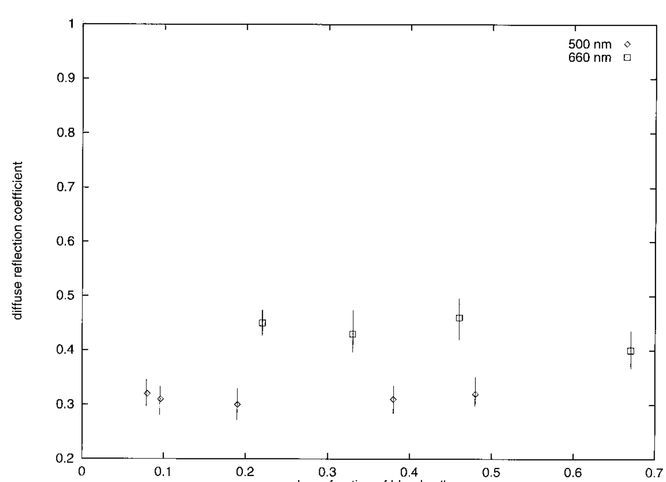

FIG. 2. The dependence of the diffuse reflection coefficient $R(1)$ on the volume fraction c of blood cells at the wavelengths 500 and 660 nm.

(1, 6, 9) and correlation effects can be ignored. Thus, correlation effects are not important for the geometrical optics large angle scattering and the denominator in Eq. [12] depends on the concentration of red cells only linearly. As has already been pointed out the dependence σ_{abs} on the concentration effects in blood can be approximately calculated with the equation

$$\sigma_{abs} = \sigma^0_{abs}(1 + c^2), \quad [13]$$

where σ^0_{abs} is the value of the absorption coefficient at $c \to 0$. The value of σ^0_{abs} is proportional to the volume fraction of red cells. Thus, from Eqs. [12], [13] it follows that

$$R(\mu_0) = R_0^q(\mu_0), \quad [14]$$

where

$$q = \sqrt{1 + c^2},\ R_0(\mu_0) = \exp\left(-4u(\mu_0)\sqrt{\frac{1 - \omega_0}{3(1 - g^{GO})}}\right) \quad [15]$$

is the reflection function of a semi-infinite weakly absorbing medium with large particles at $c \to 0$. The values of $1 - \omega_0 = \sigma^0_{abs}/\sigma^{GO}_{sca}$ and $1 - g^{GO}$ do not depend on the volume fraction of blood cells. The volume fraction c in our experiment was less than 0.7 ($q < 1.3$). Thus, the only influence of the close packing effects is decreasing the reflection function at large concentrations due to Eq. [14] ($c \approx 0.7$). One can see this trend in Fig. 2 at the wavelength $\lambda = 660$ nm.

4. CONCLUSION

We have studied the dependence of the diffuse reflection coefficient (plane albedo) of human blood at the normal light incidence on the volumetric concentration of blood cells. The dependence is weak and can be neglected in most cases. This conclusion is important for blood spectroscopy.

Note that the effect presented here is of a general interest and can be observed for other weakly absorbing optically thick media with densely packed large particles (9–11). Thus, one can arrive at the important conclusion that reflection spectra of optically thick (semi-infinite) plane-parallel layers of blood in visible light depend mostly on the geometrical parameters of red cells and the spectral behavior of their refractive index. This fact can be used for the simplification of the procedure of the retrieval of the absorption coefficient of hemoglobin in red cells. This coefficient is sensitive to the concentration of oxygen in blood, which is important for the practice of medicine.

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